H-chains have two distinct functions, namely binding (ie. to a target cell), and translocation (ie. across an endosomal membrane). The carboxy-terminal portion (H_C) of a H-chain is involved in the high affinity, neurospecific binding of the toxin to cell surface receptors, whereas the amino-terminal portion (H_N) of the H-chain is central to the translocation of the toxin into the neuronal cell. These two functions have been extensively studied and characterised, and have been mapped to distinct portions within the H-chain [see, for example, Kurazono et al (1992) J. Biol. Chem. 267, 21, pp.14721-14729; Poulain et al (1989) Eur. J. Biochem. 185, pp. 197-203; Zhou et al (1995), Biochemistry, 34, pp. 15175-15181; Blaustein et al (1987) FEBS Letts., 226, No. 1, pp. 115-120].

L-chains possess a protease function (zinc-dependent endopeptidase activity) and exhibit a high substrate specificity for vesicle and/or plasma membrane associated proteins involved in the exocytic process. L-chains from different clostridial species or serotypes may hydrolyse different but specific peptide bonds in one of three substrate proteins, namely synaptobrevin, syntaxin or SNAP-25. These substrates are important components of the neurosecretory machinery.

By way of specific example, for botulinum neurotoxin serotype A, the above functions have been mapped to amino acid residues 872-1296 for the H_C portion, amino acid residues 449-871 for the H_N portion, and residues 1-448 for the L-chain [see Lacy, D.B. & Stevens, R.C. (1999). Sequence homology and structural analysis of the clostridial neurotoxins. J. Mol. Biol. 291, 1091-1104].

All three of the above-identified domains (ie. H_C, H_N, and L) are necessary for the *in vivo* activity of a native neurotoxin, which neurotoxin may cause prolonged muscular paralysis in an affected individual. Corresponding binding, translocation, and protease functions are necessary for the *in vivo* activity of other non-cytotoxic, bacterial toxins.

It has been well documented in the art that toxin molecules may be re-targeted to a cell that is not the toxin's natural target cell. When so re-targeted, a toxin is capable of binding to a desired target cell and, following subsequent translocation into the cytosol, is capable of exerting its effect on the target cell.

For example, in the context of non-cytotoxic toxin molecules, it has been well documented that a clostridial neurotoxin may be re-targeted by incorporation of a Targeting Moiety (TM), which is not the natural TM of a clostridial neurotoxin. The described chemical conjugation and recombinant methodologies are now regarded as conventional.

In more detail, the following patent publications, in the name of the present Applicant, describe the preparation of modified bacterial conjugates.

WO94/21300 describes the preparation of modified clostridial neurotoxin molecules that, once translocated into the cytosol of a desired target cell, are capable of regulating Integral Membrane Protein (IMP) density present at the cell surface of the target cell. The modified neurotoxin molecules are thus capable of controlling cell activity (eg. glucose uptake) of the target cell.

WO96/33273 describes the preparation of modified clostridial neurotoxin molecules that target peripheral sensory afferents. Once delivered into the cytosol of a peripheral sensory afferent, the modified neurotoxin molecules are capable of demonstrating an analgesic effect.

WO98/07864 describes the preparation of single chain, modified clostridial neurotoxin molecules, which single chain molecules are substantially inactive in terms of sequential binding, translocation and L-chain dependent endopeptidase activities. The single chain molecules are activatable into active di-chain molecules through a proteolytic cleavage reaction.

WO99/17806 describes the preparation of modified clostridial neurotoxin molecules that target primary sensory afferents, which modified neurotoxins are capable of demonstrating an analgesic effect.

WO00/10598 describes the preparation of modified clostridial neurotoxin molecules that target mucus hypersecreting cells (or neuronal cells controlling said mucus hypersecreting cells), which modified neurotoxins are capable of inhibiting hypersecretion from said cells.

WO01/21213 describes the preparation of modified clostridial neurotoxin molecules that target a wide range of different types of non-neuronal target cells. When so targeted and delivered into the cytosol, the modified molecules are capable of preventing secretion from the target cells.

Additional publications in the technical field of re-targeted toxin molecules include:- WO00/62814; WO00/04926; US5,773,586; WO93/15766; WO00/61192; WO99/58571; and US2003/0059912.